EFFECT OF LEAD INGESTION ON IN VITRO STEROID SYNTHESIS OF THE RAT ADRENAL GLAND[1]

G. L. WRIGHT,[2] M. A. LESSLER, AND S. G. IAMS[3]

Department of Physiology, Ohio State University College of Medicine, Columbus, Ohio 43210

WRIGHT, G. L., M. A. LESSLER AND S. G. IAMS. Effect of lead ingestion on *in vitro* steroid synthesis of the rat adrenal gland. Ohio J. Sci. 75(3): 155, 1975.

The addition of 1% lead acetate to the diet of male Sprague-Dawley rats caused a stimulation of the adrenal gland. Blood lead concentration rose to 85 μg/dl in one week and stayed at or above this level for the eight-week experimental period. Lead ingestion resulted in an increase in adrenal weight during the first four weeks of lead exposure, followed by a return to near control values. The net-percent conversion of ^{14}C-progesterone by adrenal tissue to 11-deoxycorticosterone, aldosterone, and deoxycortisol first rose, then returned to near or below control values, in a variable pattern, by the eighth week. The data suggest an initial stimulation of the adrenal gland function during the early stages of lead intoxication followed by either an adaptation or an exhaustion of adrenal.

Although the recent increased awareness of the problems posed by lead in the

[1]This investigation was supported in part by the International Lead Zinc Research Organization and General Research Support Grant 5409, National Institutes of Health. Manuscript received March 12, 1975, revised June 9, 1975 (75-16).

[2]Present Address: National Institute of Occupational Safety and Health, Division of Laboratories and Criteria Development, Physiology and Ergonomics Branch, 1014 Broadway, Cincinnati, Ohio 45202.

[3]Present Address: May Institute for Medical Research, Cincinnati, Ohio 45229.

environment has resulted in investigations of this element's influence on a wide range of biological functions, relatively little is known concerning its effect on the adrenal gland. Hypertrophy and hyperplasia of the adrenal cortex has been observed in lead exposed rodents (Vályi-Nagy *et al.*, 1954; Nurmaganbetov, 1962a). Nurmaganbetov (1962b) has examined ketosteroid formation of the adrenal cortex in lead acetate injected rabbits and concluded that the early effect of lead on secretory activity was stimulatory in nature while extended exposure resulted in necrosis and depletion of lipids and ketosteroids. Additional evidence of a deleterious effect of lead on adrenal function was reported by Makotchenko (1970) who observed a decrease in the adrenal response to ACTH injections in chronically lead poisoned humans. Earlier, Nakade (1958) was unable to show a definite change in the fluorogenic corticord content of plasma and adrenal glands of rats injected with lead salts. In particular, there have been few if any studies of steroidogenesis during intoxication by lead ingestion. This present study was done to evaluate the *in vitro* steroid synthesis of rat adrenals obtained from animals during the early phases of chronic lead intoxication.

MATERIALS AND METHODS

Male Sprague Dawley rats, weighing approximately 335 g at the start of the experiment, were housed three per cage in rooms maintained at 23°±1°, with a 12 hour light, 12 hour dark photoperiod. Control animals received ground Purina Chow and the experimental animals were fed the same diet containing 1% lead acetate. Experimental and control animals were allowed a 5-day period to adjust to handling and the ground Purina Chow before 1% lead acetate was added to the food of the experimental animals.

The blood lead content of control and lead fed animals was determined at weekly intervals by the nitric acid-wet ash modification of the Delves Cup Atomic Absorption technique (Barthel *et al.*, 1973). Blood samples for the lead determinations were obtained in heparinized capillary tubes from small incisions of the tail vein or from the severed neck vessels on days that the animals were sacrificed.

In vitro steroid synthesis was determined for lead exposed rats at weekly intervals during an eight-week period. Lead-fed and control animals were sacrificed by decapitation in groups and each data point is based on the average of 3 animals. The adrenal glands were cleared of adherent tissue, weighed, and prepared for incubation by mincing and suspension in a Krebs-Ringer's bicarbonate solution containing glucose, glucose-6-phosphate, nicotinamide, sodium fumarate, NAD, NADH, NADP, and ATP as co-factors (Brownell, 1957). Approximately 50 μg of ^{14}C-progesterone per 100 mg of adrenal tissue was added to each flask as the steroid precursor just prior to the three-hour incubation in a Dubinoff shaker at 37°C under a 95% O_2 and 5% CO_2 atmosphere. The incubant was extracted three times with ethyl acetate and the metabolites separated by the method described by Lucis *et al.* (1965) utilizing paper chromatographic techniques. Identification procedures included comparison of the relative mobilities of the various compounds and their oxidized and acetylated forms with those of authentic cold standards. The position of the metabolites on the paper was determined with an RCL paper strip radioactivity scanner. Areas exhibiting radioactivity were cut into small fragments and eluted for quantitation with a dual channel Tri-carb Liquid Scintillation Spectrophotometer. The data were fitted by computer to both linear and quadratic equations, and the appropriateness of the equation and statistical significance were determined by t-test and the R-square method for measure of fit (Sokal and Rohlf, 1969).

RESULTS

Lead ingestion caused a rapid elevation of the blood-lead to levels normally associated with lead poisoning. The blood lead concentration of the control groups remained relatively constant at approximately 25 μg per 100 ml of whole blood during the eight week experimental period. As expected, the lead-fed rats showed increased blood-lead levels (75–85 μgPb/dl) during the first five weeks and had mean blood lead values between 85 and 125 μg/dl during the remaining three weeks of the study.

Lead ingestion resulted in a marked but variable pattern of change in the adrenal gland to body weight ratio and the *in vitro* synthesis of 11-deoxycorticosterone, aldosterone, and deoxycortisol. The change in adrenal weights and the *in vitro* synthesis of these three steroids was found in each case to be significantly different for the control and lead-fed groups during the 8 week experimental period. Mean adrenal gland weight per 100 g body weight in the lead-fed animals was 13% to 17% more than the controls during the first 4 weeks of lead ingestion (fig. 1). During the latter 4 weeks of

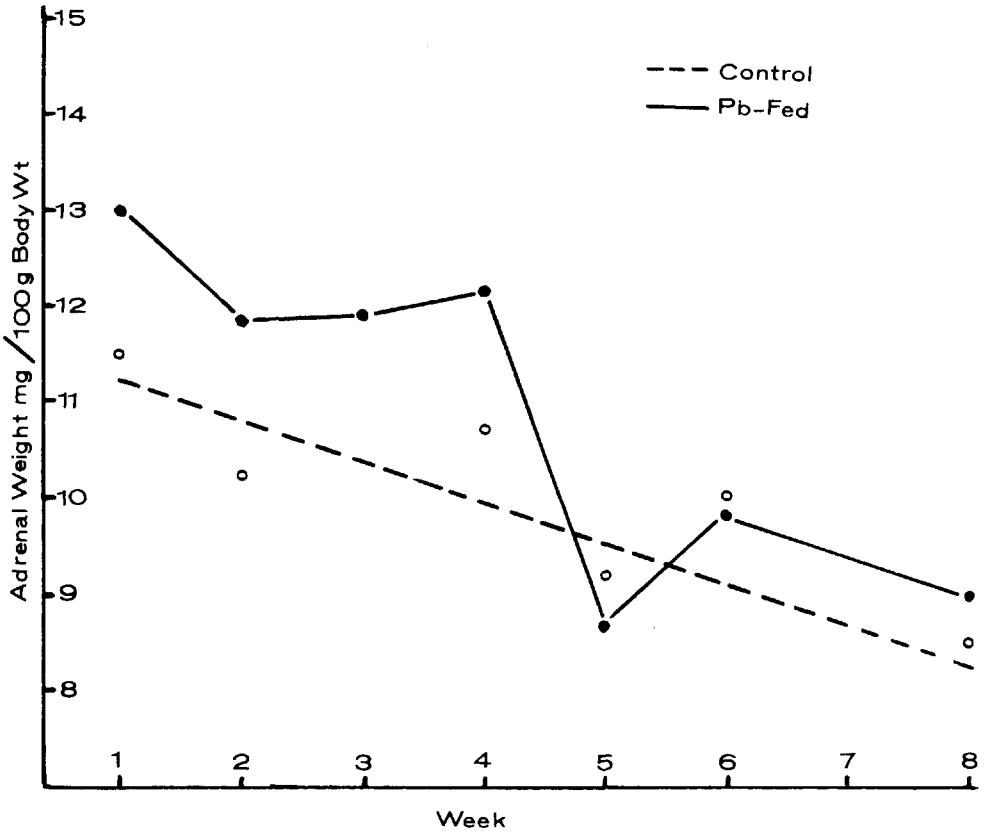

FIGURE 1. Comparison of adrenal gland weights of control and lead-fed rats. The closed circles show mean adrenal gland weights of lead-fed animals. The dashed line is the best fit linear equation for the control data (open circles). Each point is the average of three animals.

the 8 week period, the adrenal weights returned to near normal control values.

Control net-percent conversion of ^{14}C-progesterone to 11-deoxycorticosterone increased from 6.0% for the first week to 12% at the eighth week, while a progressive decrease was noted with adrenals from the lead-fed rats (fig. 2). The net-percent conversion to deoxycorticosterone obtained for adrenal tissue from lead-fed rats was elevated above control values during the first week (18%), but returned to approximately control levels during weeks 3 through 5, then decreased to well below control levels during the last three weeks of the study. A low net-percent conversion (10 times less than controls) value of 0.3% was found the eighth week with adrenal gland tissue from lead-fed rats.

In vitro aldosterone synthesis did not differ from control levels during the first week of lead ingestion (fig. 3). Aldosterone synthesis, however, showed a four-fold increase the second of lead intoxication, and remained elevated during the succeeding four weeks, then fell, and reached approximately control levels the seventh and eighth weeks. The maximum value of 1.6% conversion (slightly in excess of 10 times the average control value) was found during the third week and remained about six times above control levels until the last two weeks of the study.

Adrenals from the lead-fed animals showed increased net-percent conversion of progesterone to deoxycortisol (fig. 4) in a pattern somewhat similar to that observed for aldosterone. *In vitro* pro-

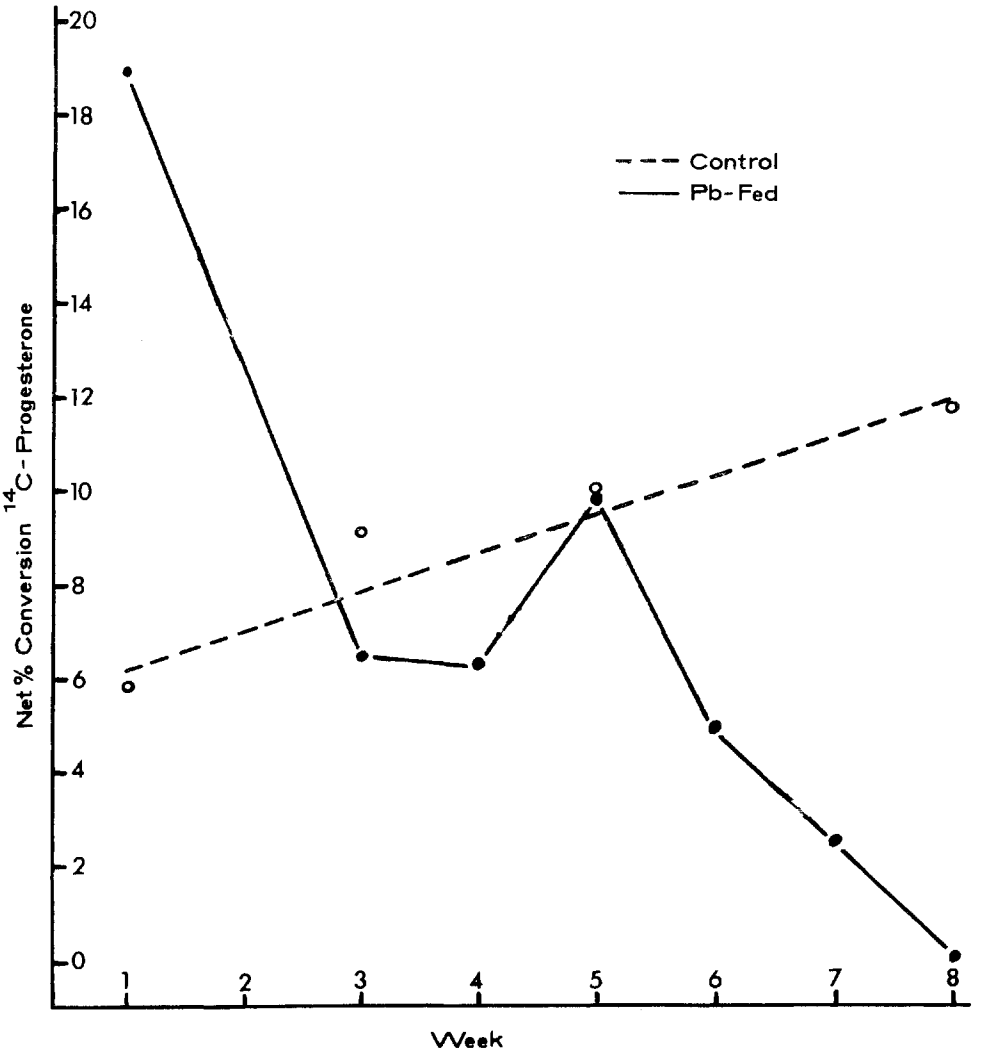

FIGURE 2. Conversion of ^{14}C-progesterone to 11-deoxycorticosterone by adrenal glands obtained from control and lead-fed rats. The closed circles show *in vitro* 11-deoxycorticosterone production by adrenal glands from lead-fed animals. The dashed line is the best fit linear equation for the control data (open circles). Each point is the average of three animals.

duction of this steroid showed a 2.5 to 4.5-fold increase above the average control values during the first five weeks with maximum values of 3.5 to 3.9% conversion recorded during the second and third weeks. The synthesis of deoxycortisol in the lead-exposed adrenal tissue did not appear to differ from the control levels during the last two weeks of the study.

DISCUSSION

Rats with 1% lead acetate added to their food showed three to five-fold increases in their blood-lead in two to three days (Cardona and Lessler, 1974). Blood lead levels above 75 μg/dl (observed by us after one week) coupled with retardation in growth and hematologic changes, are considered indicative of lead intoxication (Hernberg *et al.*,

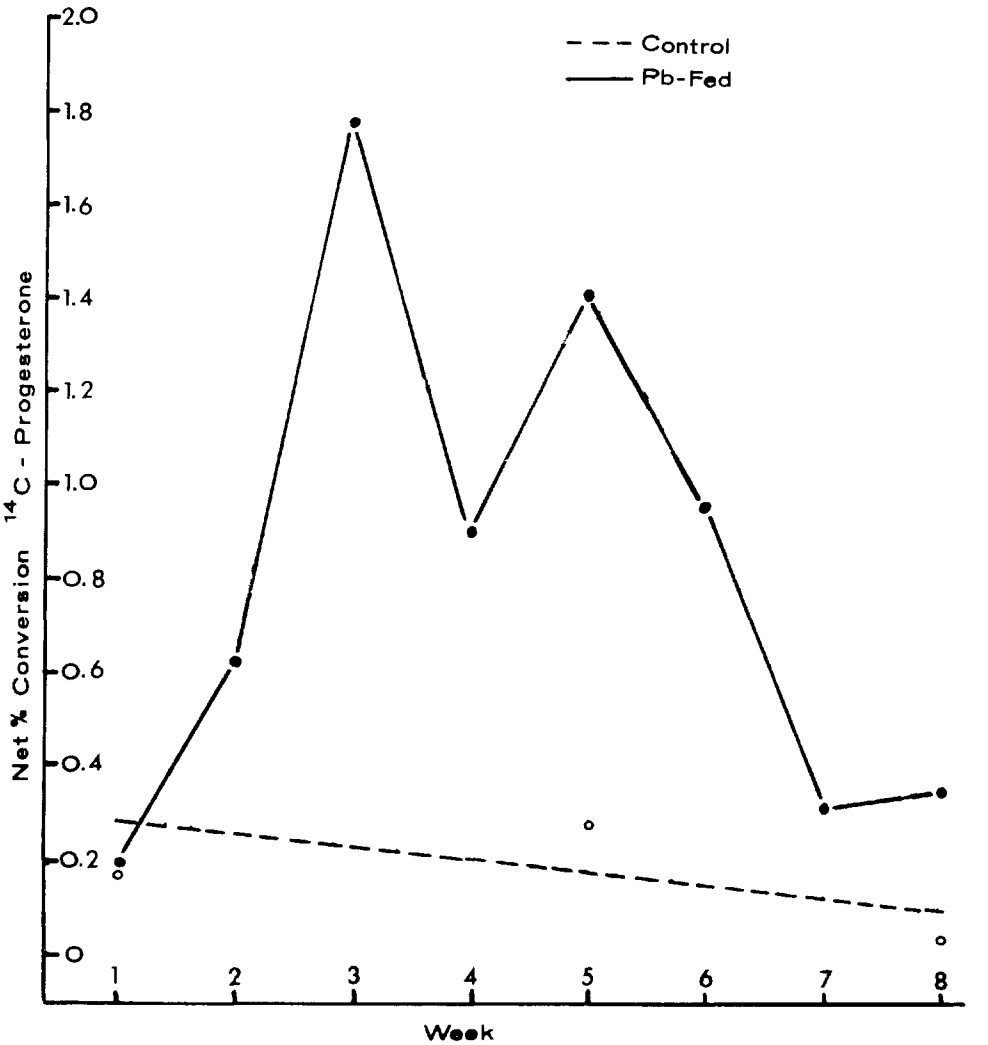

FIGURE 3. Conversion of ^{14}C-progesterone to aldosterone by adrenal glands obtained from control and lead-fed rats. The closed circles show *in vitro* aldosterone production by adrenals from lead-fed animals. The dashed line is the best fit linear equation for the control data (open circles). Each point is the average of three animals.

1970). The reasons for fluctuations in blood lead concentration which we observed during the last three weeks of the study are not known. Cardona and Lessler (1974) suggest the possibility that alterations in the pattern of lead mobilization and/or excretion which occur during an extended period of lead exposure may result in fluctuations in blood lead concentrations.

The 13% to 17% increase in adrenal gland to body weight ratio we observed during the first four weeks of lead ingestion (fig. 1) suggests adrenal hypertrophy due to stimulation by the lead added to the diet. In contrast, the later decrease in adrenal gland to body weight ratio to control levels during the last four weeks may be indicative of either an adaptation or exhaustion of the gland following the hypertrophy.

Lead exposure resulted in an initial

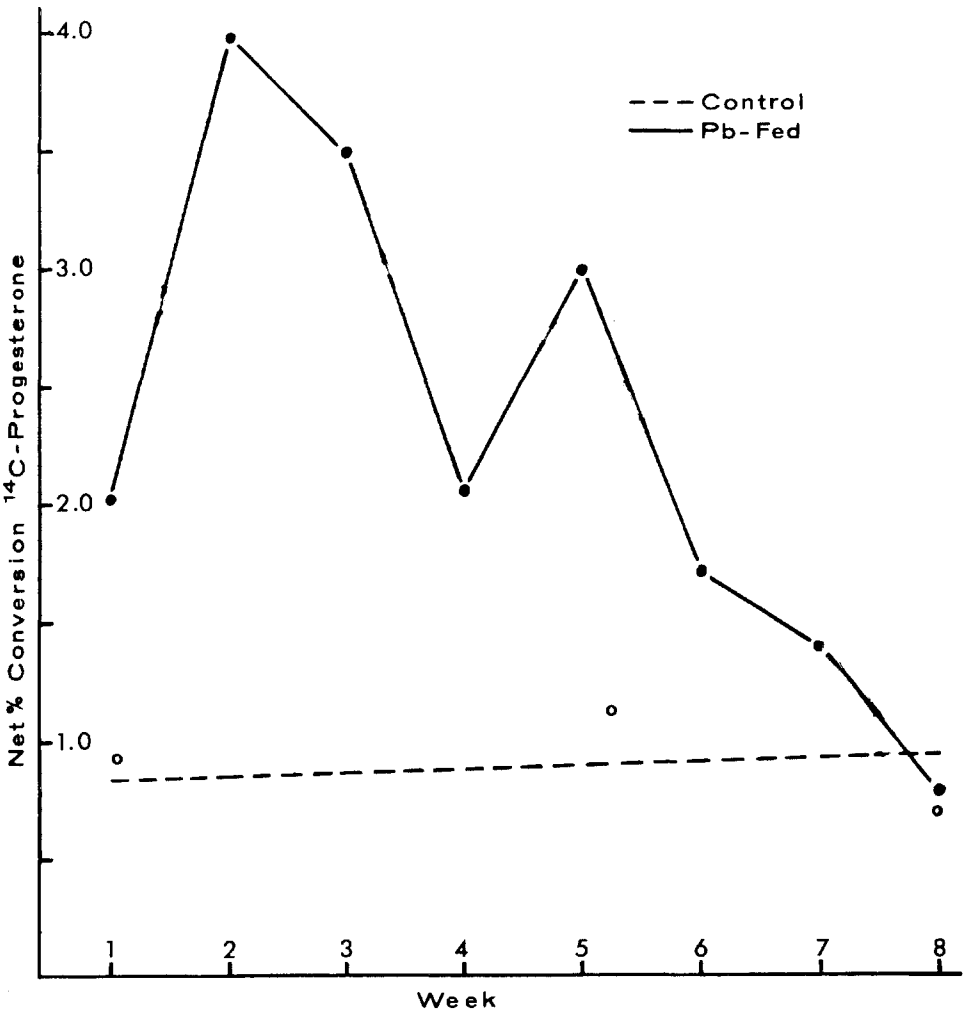

FIGURE 4. Conversion of ^{14}C-progesterone to deoxycortisol by adrenal glands from lead-fed animals. The dashed line is the best fit linear equation for the control data (open circles). Each point is the average of three animals.

increase and subsequent decrease in the synthesis of 11-deoxycorticosterone by the rat adrenal gland (fig. 2). However, in view of the observed increase in the synthesis of the other steroids examined, the apparent decrease in deoxycorticosterone production may be consistent with a stimulation of the adrenal cortex that reflects an increase in the activity of the enzyme systems utilizing deoxycorticosterone as an intermediate.

The observed increase in aldosterone production in lead-exposed rats (fig. 3) concurs with the findings of Altamura *et al.* (1971) who showed an increase in the urinary aldosterone output in lead poisoned rabbits. At the present time the effect of lead on electrolyte metabolism is incompletely understood, but reports of changes in tissue sodium and potassium concentrations suggest that electrolyte imbalance may be a factor in lead poisoning. This may be associated with a lead induced increase in membrane ionic permeability, or a decrease in the Na^+–K^+ pump activity (Makashev and

Kazachenko, 1968). It is possible that the elevation in aldosterone synthesis observed in our study represents an adrenal cortex response to a lead induced alteration in blood sodium concentration in addition to or in the absence of a direct effect of lead on the enzyme systems associated with synthesis of aldosterone.

It was generally believed that the rat adrenal does not synthesize deoxycortisol, but Brownell and coworkers (1963) isolated this steroid during the *in vitro* incubation of adrenals from hypertensive rats. Earlier, Boulouard (1957, 1959) reported hydroxycorticosteroid production in studies with cold stressed and thyroidectomized rats, suggesting that pathways for the formation of deoxycortisol may be present, although not generally active in the rat. The significance of the increase in deoxycortisol synthesis is uncertain, and the present data do not provide information concerning the specificity of the response of increased activity in synthesizing this steroid during lead poisoning.

It has become apparent that lead intoxication in mammals exerts a significant influence on a wide array of biological parameters. Our present lack of knowledge concerning the direct and indirect effects of lead poisoning on endocrine function belies the importance of this system in the normally functioning or stressed animal. Our data, which indicate adrenal gland involvement in chronic lead intoxication, may be important in the complete evaluation of the effect of lead exposure on an otherwise intact animal.

Acknowledgment. The authors would like to thank Mary Ann Decker for technical assistance during the preparation of the mansucript.

LITERATURE CITED

Altamura, B., A. Chiantera, A. Lo Storto, F. DeSalvia, N. L'Abbate and M. Husti. 1971. L'escrezione urinaria dell' aldosterone nel salurnismo sperimentale. Medicina del Lavora 62: 472–477.

Barthel, W. F., S. L. Smrek, G. P. Angel, J. A. Liddle, P. J. Landigran, S. H. Gehlbach and J. J. Chisolm. 1973. Modified delves cap atomic absorption determination of lead in blood. J. Assoc. Offic. Anal. Chem. 56: 1252–1256.

Boulouard, R. 1957. Action du froid sur la teneru en 17-hydroxycorticosteroids du sang chez le rat et la cobaye. Compt. Rend. Soc. Biol. (Paris) 151: 913–917.

——— 1959. Action due froid sur la teneru en 17-hydroxycorticosteroids du plasma chez le rat normal et tyroidectomize. Compt. Rend. Soc. Biol. (Paris) 153: 203–205.

Brownell, K. 1957. Steroid production of the normal opossum adrenal *in vitro*. Gen. Comp. Endo. 9:214–216.

———, S. L. Lee, R. R. Beck and P. K. Besch. 1963. In vitro 17 alpha hydroxylation of steroids by the adrenals of hypertensive rats. Endocrinol. 72: 167–168.

Cardona, E. and M. A. Lessler. 1974. Time course of hematologic changes during chronic lead poisoning. Proc. Soc. Exp. Biol. Med. 145: 663–668.

Hernberg, S., S. Nikkanen, G. Mellin and H. Lilius. 1970. d-aminolevulinic acid dehydratase as a measure of lead exposure. Arch. Environ. Health. 21: 140–145.

Lucis, R., A. Carbollerin and E. H. Veming. 1965. Biotransformation of progesterone-4-C^{14} and deoxycorticosterone-4-C^{14} by rat adrenal glands *in vitro*. Steroids. 6: 737–756.

Makashev, K. K. and L. V. Kazachenko. 1968. Disturbances in electrolyte balance during lead poisoning. Izvestiya Akademii Nauk. Kazakhskoi SSR Seriya Biologicheskaya. 6: 64–68.

Makotchenko, V. M. 1970. The functional condition of the hypothalamic-hypophyseal-adrenal system in chronic industrial poisoning. Endokinopatii lech. Gorm. Resp. Mezhved. Sb. 5: 80–88.

Nakade, R. 1958. The content of fluorogenic corticoids in plasma and adrenal gland of the rat intoxicated with various metals. Nippon Naibumpi Gakkae Shi. 34: 131–147.

Nurmaganbetov, E. K. 1962a. Distribution of lipids and ketosteroids in the adrenal cortex in the course of lead intoxication. Trudy Inst. Kraevoi Patologii, Akademiya Nauk. Kazakhskoi SSR. 10: 121–127.

——— 1962b. Changes in the adrenal cortex in acute lead poisoning. Vestnik Akademii Nauk. Kazakhskoi SSR. 18: 88–95.

Sokal, R. R. and F. J. Rohlf. 1969. Biometry, W.H. Freeman, San Francisco.

Vályi-Nagy, T., L. Kocsar, B. Kelentey, L. Keszytus, H. Csernyáncky, L. Kertesz and S. ÖKrös. 1954. Experimental lead poisoning. Kiserletes Orvostudomany. 6: 124–137.